Methanol Institute

Comments on the U.S. EPA Draft Toxicological Review of Noncancer Effects of Methanol (IRIS)

Docket ID NO. EPA-HQ-ORD-2009-0398

June 17, 2011

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EXECUTIVE SUMMARY

EPA has published a hazard assessment of the noncancer effects of methanol for public comment and review. In that assessment, EPA has recommended reference levels (an oral reference dose and an inhalation reference concentration) above which exposure to methanol may pose a risk of noncancer effects in humans. In these comments we reiterate the reasons why we believe that these reference levels cannot be justified on the basis of the scientific evidence.

There are several reasons why we believe these recommended reference levels are not correct:

1. EPA has arbitrarily decided to establish these reference levels to identify risks ONLY for exposure to methanol that increases the body burden of methanol or its metabolites.

In doing so, EPA has implicitly declared that whatever a person's body burden of methanol is, this level of methanol is safe, but that potentially small increments over that level are toxic. People naturally create methanol in their bodies, and exposure to external methanol is not required. EPA has presented no evidence regarding what level of body burden is just below the level that poses a risk, such that a little more methanol in addition to this body burden potentially poses a risk. This ignoring of the background levels in blood is especially problematic because the body burden of the general population varies over a very wide range, from 0.25 mg/L to 4.7 mg/L. of blood, according to EPA's assessment.

2. The recommended reference levels represent a very small addition to the average person's body burden of methanol and by implication suggest that half the population is at risk from their own background level of methanol.

By our calculations, exposure to a level of methanol equal to the reference level would increase one's body burden by only 1% to 16%, depending on whether one had a high body burden or a low body burden within the range. The increase of 16% comes from adding 0.04 mg/L to a background level of 0.25 mg/L, for a total of 0.29 mg/L. Such a 16% increase in blood methanol might theoretically represent a difference sufficient to raise a concern under EPA's reasoning

about increased developmental risk. However, if a blood methanol above 0.29 mg/L poses a developmental risk, then almost all people are at risk from their own background level of methanol. EPA calculates a higher blood level that would be caused by exposure at the reference levels, but even using EPA's figures, a person at the median background level of 2.0 mg/L would have an increment of only 18%. Therefore, even with EPA's higher figures, half of the population would thereby be at risk from their own background level of methanol. EPA presents no epidemiological or other substantiation that such a large population is at risk from methanol from any source.

3. EPA incorrectly supports its decision to ignore the naturally-occurring background levels of methanol in human blood by citing the results of its PBPK modeling.

The Agency found that the results of modeling the rat exposures did not vary more than 1% no matter whether the background level in the rats' blood was considered or not. We do not believe this analysis addresses the critical problem of ignoring background levels in humans for the following reason. The background level of methanol in the blood in the test rats was a very small percentage of the large dose of methanol used in the test. Therefore, it is easy to see why the results from the PBPK modeling of the rats would be approximately the same whether background levels were included or not. However, in the case of humans with a wide range of background blood levels and the exposure to the small increment of methanol representing the recommended reference level, the background levels in humans are not a small percentage of the TOTAL blood level of methanol after exposure. Therefore, the PBPK modeling of the rats has given EPA false confidence that they can legitimately ignore the background levels of methanol in humans.

4. While the increment of a reference level dose of methanol is a small percentage of the average background blood level in humans, the intake of certain common foods can easily exceed EPA's recommended reference level.

While information about methanol levels in foods is not extensive, we conclude from the available evidence that a child's consumption of a glass of pasteurized orange juice could cause that child to exceed the reference dose that EPA recommends.

5. The implications of the EPA assessment, then, are that most Americans are already at risk of developmental effects from their naturally-produced methanol, even without any exposure to external methanol, and that the consumption of a glass or two of pasteurized orange juice or some other foods will put them at even greater risk.

We believe that these implications are sufficient to cause EPA to step back and take a very serious look at the basis of its conclusions that such low levels of methanol are hazardous, given the lack of any other evidence to support these risk estimates in humans.

6. In addition, recent research by Dr. Peter Wells of the University of Toronto, which we detail in these comments, raises serious questions about the use of rodent models for hazard assessment of methanol in humans because rodents and humans metabolize methanol very differently.

EPA's current recommended reference levels rest entirely upon rodent data, and as a consequence of Dr. Wells' research, EPA needs to reassess the basis for its conclusions on the noncancer effects of methanol.

From the above points, it is clear that our criticism of the EPA assessment goes to the fundamental bases of the assessment and not to peripheral matters. If EPA were to consider background levels of methanol and/or were to change the study on which the reference levels are based, the assessment would have to be radically changed. We have urged EPA to reassess its approach to calculating the risk of methanol in humans and to make the necessary changes to the assessment BEFORE completing public comment and peer review; however, should EPA decide otherwise, then once these changes are made, a completely new review cycle will be necessary to ensure the integrity of the scientific process. Otherwise, EPA will make all the key decisions AFTER peer review—an approach inconsistent with its commitment to peer review of major decision documents.

Should EPA proceed to peer review without changing the document, as EPA has recently indicated it intends to do, EPA should at a minimum address these fundamental criticisms in a written document. That document should be provided to the public for written comment by Dr. Wells and other outside scientists with sufficient lead time so that these written comments can be placed before the peer reviewers BEFORE they are required to draft their initial comments on the assessment document. (Under EPA procedures, these draft comments from the reviewers must be submitted 10 days before the peer review panel meeting, and hence their drafting must begin long before then.). In addition, more detailed and focused charge questions need to be constructed and presented to the peer review panel to ensure that these critical issues are addressed adequately by the panel.

We urge EPA not to rush ahead to the peer review of an incomplete document, but to pause and address these issues fully in its assessment effort BEFORE peer review of the assessment.

Methanol Institute's Comments
On the U.S. EPA
Draft Toxicological Review of Noncancer Effects of Methanol (IRIS)

I. About Methanol and the Methanol Institute

Methanol is one of the most widely used chemicals in commerce, with annual global consumption of nearly 16 billion gallons. Methanol is a basic building block for hundreds of chemical compounds and products that touch our daily lives. Methanol is also an emerging energy fuel, with the potential to fuel our vehicles, electric power plants, homes and consumer electronics.

As the trade association for the global methanol industry, the Methanol Institute represents the world's leading methanol producers, distributors, and technology companies. Since the EPA began its assessment of methanol, the Institute has worked cooperatively with EPA staff to assist the Agency in obtaining the best available science for its assessment. The Methanol Institute:

- provided a large number of research documents to EPA via the IRIS Submission Desk;
- initiated a four-year research project with the University of Toronto to address a basic research question related to the metabolism of methanol that was identified as needed by EPA staff;
- obtained the original research data from Japan for the NEDO studies on methanol, and translated and provided this information to EPA;
- engaged an expert modeler to review the PBPB model employed by the EPA in its methanol assessment; and
- initiated a research project to determine the role of formaldehyde in the metabolism of methanol.

II. BACKGROUND

In June 2010, EPA put the IRIS assessment of methanol on hold due to concerns raised regarding the underlying data relied on for a cancer assessment. In April 2011, EPA announced that it would continue to hold the cancer assessment of methanol, but would proceed with a noncancer assessment. Unfortunately, EPA chose to ignore comments submitted on the noncancer sections of the 2009 draft assessment and any new research published since 2007. Rather than revising the draft assessment based on an evaluation of the comments received and new research, EPA

reissued the same draft (with the cancer section deleted). Thus the peer reviewers are being asked to comment on a draft that we believe is not up-to-date, avoids mention of key scientific research performed between 2007 and 2011, contains errors in science and numbers, as well as incorrect assumptions regarding the proper model from which to draw conclusions regarding human health effects of methanol. The 2011 noncancer assessment of methanol suffers from many of the same issues raised in the National Academy of Sciences (NAS) review of the draft IRIS file on formaldehyde. It contains long detailed discussions of studies not central to the assessment, lacks clear justification for the choice of endpoint and critical studies, and asserts there is a difference between methanol generated endogenously and methanol from external sources such as drinking water or air. This assessment needs to be re-done (and subjected to new peer review) to address endogenous methanol in relation to toxicity and metabolism and the relevance of rodent developmental toxicity in light of more recent mode of action research publications.

III. EPA ASSUMES ENDOGENOUS METHANOL IS SAFE AND THAT EXOGENOUS METHANOL IS TOXIC

The EPA draft assessment implicitly assumes that endogenous (background) methanol is safe no matter how much is present, but that tiny increases of exogenous (intake from external sources) methanol are potentially toxic. This is equivalent to declaring that the dermal exposure to a chemical is toxic but that the simultaneous inhalation of the chemical is not toxic without demonstrating the differences in the two exposures that would account for the difference in the toxicity.

In the case of endogenous and exogenous methanol, EPA has not postulated a difference in distribution or other characteristic that would explain their assumed difference in toxicity. We believe good toxicologic practice requires that endogenous and exogenous exposures be considered as toxicologically equivalent unless there is evidence to the contrary.

We include here statements from the draft assessment in order to demonstrate EPA's position on this subject, followed by our response.

III. A. EXCERPTS FROM EPA'S DRAFT IRIS DOCUMENT MAKE EPA'S APPROACH CLEAR

#1

"The primary purpose of this assessment is for the determination of noncancer risk associated with exposures that increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde) above prevailing, endogenous levels. Thus, the focus of model development was on obtaining predictions of increased body burdens over background following external exposures. To accomplish this, the PBPK models used in this assessment do not account for background levels of methanol, formaldehyde or formate. In addition, background levels were subtracted from the reported data before use in model fitting or validation (in many cases the published data already have background subtracted by study authors). This approach for dealing with endogenous background levels of methanol and its metabolites assumes that: (1) endogenous levels do not contribute significantly to the adverse effects of methanol or its metabolites; and (2) the exclusion of endogenous levels does not significantly alter PBPK model predictions. There is uncertainty associated with these assumptions." (p. 3-27)

#2

"As described in Section 3.4.3.2, the focus of model development is on obtaining accurate predictions of increased body burdens over endogenous background levels of methanol and its metabolites. The PBPK models do not describe or account for background levels of methanol, formaldehyde or formate." (p. 5-7)

#3

"As described in Chapter 3, background levels of methanol and its metabolites are produced through endogenous metabolic processes. Potential risks resulting from these endogenous levels are not determined in this IRIS assessment. This assessment focuses on the determination of noncancer risk associated with exogenous methanol exposures that increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde) above endogenous background levels. Average background blood levels in healthy adults following restriction of methanol-producing foods from the diet are reported in Section 3.1 (Table 3-1). The mouse, rat and human PBPK models developed for this assessment predict increased blood levels of methanol and its metabolites over background following oral or inhalation exposure to methanol (see further discussion in Section 3.4.3.2). Consequently, this assessment provides estimates of noncancer risk from oral and inhalation exposures above sources of methanol that contribute to background blood levels." (p. 6-3)

#4

"Given the reactivity of formaldehyde, models that predict levels of formaldehyde in the blood are difficult to validate. However, production of formaldehyde or formate following exposure to methanol can be estimated by summing the total amount of methanol cleared by metabolic processes.[10] This metric of formaldehyde or formate dose has limited value since it ignores important processes that may differ between species, such as elimination (all routes) of these two metabolites, but it can be roughly be equated to the total amount of metabolites produced and may be the more relevant dose metric if formaldehyde is found to be the proximate toxic moiety. Thus, both blood methanol and total metabolism metrics are considered to be important components of the PBPK models. Dose metric selection and MOA issues are discussed further in Sections 3.3, 4.6, and 4.8. "(p. 3-17,18)

III. B. OUR RESPONSE TO EPA'S APPROACH

One cannot assess the toxicity of exogenous methanol as a separate issue from endogenous methanol. It is scientifically unsound to assume that endogenous methanol (no matter how much) does not cause toxicity but that exogenous methanol (at very low levels) carries a toxicological risk. However, this is precisely what the IRIS draft assessment attempts to do.

It is well understood that toxicity is caused by the total concentration of a chemical reaching the target organ, whether via the parent compound or a metabolite distributed to the organ or generated *in situ*. One cannot separate the toxicity from endogenous methanol from that of exogenous, unless there is a difference in distribution within the body or other distinguishing factor. The EPA draft assessment provides no evidence that such factors differentiate exogenous and endogenous methanol. Comments from both the Department of Defense on the Draft Methanol IRIS file (dated Nov. 23, 2009) and the NAS review of the Formaldehyde IRIS file[1] indicate that background/endogenous levels of exogenous chemicals should be included in any evaluation.

III. B. 1. THE RECOMMENDED REFERENCE DOSE IS ONLY A FRACTION OF THE BACKGROUND LEVEL OF METHANOL

The assessment asserts its objective is to determine "the noncancer risk associated with exposures that increase the body burden of methanol or its metabolites". However, the assessment recommends a reference dose that represents only a fraction of the background level of methanol in the general public. Exposures at the reference dose level are therefore

[1] National Research Council, National Academy of Sciences, Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde, 2011, Summary, page 3; Toxicokinetics, page 23.

not likely to increase the body burden of methanol or its metabolites. As is discussed later in these comments, EPA's choice of such a low reference level suggests, by implication, that perhaps half or more of the population is at risk from their own background level of methanol.

The PBPK model used in the methanol assessment assumes that endogenous methanol has no impact on the metabolism of exogenous methanol. In developing the model, EPA attempted to subtract the endogenous methanol. The background human blood level of methanol ranges from 0.25 to 4.7 mg/L (Table 3-1 of EPA's assessment), and the PBPK model uses 2.0 mg/L as the background level of methanol[2].

The document recommends an RfD of 0.4 mg/kg/day and an RfC of 2 mg/m^3, but does not demonstrate that these exposures increase the body burden of methanol or its metabolites. Based on EPA's PBPK model, exposure to the recommended RfD (as 6 bolus events over 12 hours) would result in peak blood methanol of 0.08 mg/L. Exposure for 24 hours to the recommended RfC would result in a blood methanol level of only 0.04 mg/L[3] These exposure levels would constitute only a small percentage of the background/endogenous levels.

Based on Table B-5, it appears that the model predicts human exposure to exogenous methanol would only exceed background blood levels of methanol when the exposures are greater than 40 mg/kg/day orally[4] or 100 ppm via inhalation[5]. There is no discussion in the assessment of what exposure to exogenous methanol would increase the blood level of methanol beyond background, or whether exposure to exogenous methanol is as toxic to persons with a low background level (0.25 mg/L) as to those with a high background level (4.7 mg/L). At the high end of the endogenous methanol range, this exogenous exposure at the RfC level would change the total blood methanol from 4.7 to 4.74 mg/L. It is hard to imagine that this 1% increase in blood methanol would result in increased developmental risk. At the low end of the endogenous range, exogenous exposure at the RfC level would change the total blood methanol from 0.25 to 0.29 mg/L (a 16% increase). A 16% increase in blood methanol might theoretically represent a difference sufficient to raise a concern about increased developmental risk. However, if a blood methanol above 0.29 mg/L is a developmental risk, then almost all people are at risk from their own endogenous methanol.

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[2] At page B-93, CVBBG is shown to be the variable used to set the background concentration of methanol; CVBBG is set to "2" in the model's data file, human_"inh_sim.m". At the Methanol listening session on May 26, 2011 EPA scientists stated that they did not use a fixed value for the background level in the model. However, the PBPK file indicates they did.

[3] These calculations are drawn from a report prepared for the Methanol Institute by Dr. Teresa Leavens, Center for Chemical Toxicology Research and Pharmocokinetics, North Carolina State University (Appendix B)

[4] The EPA PBPK model predicts a blood level of 0.83 mg/L from oral exposure to 10 mg/L and 5 mg/L from 50 mg/kg/day.

[5] The EPA PBPK model predicts a blood level of 2.64 mg/L from inhalation exposure to 100 ppm.

In a letter to the Methanol Institute dated May 27, 2011[6], EPA's Dr. Rebecca Clark responded to some earlier expressions of this concern for the relationship of the reference levels to background levels in humans expressed by the Methanol Institute. In her letter, Dr. Clark indicated that for individuals of the population at the lower end of the background level range (0.25 mg/L), exposure to the reference levels of methanol are predicted by the PBPK model to raise methanol blood levels by more than 150% over their background levels.

We assume by this that EPA calculated an added blood level of 0.37 mg/L (0.25 * 1.5). Two independent calculations of blood methanol levels from exposures at the reference concentrations (by Juice Producers' previously submitted comments at less than 0.05 mg/L and the Methanol Institute's comments at the EPA Listening Session at less than 0.08 mg/L) are significantly lower than EPA's assertion of the level produced using the same PBPK model as included in the draft IRIS assessment. Clearly there is some confusion on exactly what the addition of the exposure to the reference levels would mean for blood levels of methanol.

However, even taking the figures presented in Dr. Clark's letter regarding the resulting blood level, we calculate that the exogenous contribution of a reference dose level to the overall blood level would vary from 150% at the lower end of the population background level range (which Dr. Clark cites) to 18% at the median point in that range (2 mg/L), to 8% at the highest point in the range. In other words, for most Americans, an exogenous exposure to methanol at the reference level would contribute only a small percentage to their methanol blood level, raising questions of whether EPA has in fact accurately identified a level above which a person is at risk from methanol exposure.

But at the same time, if EPA is correct in its recommended reference level, a person is at risk of reproductive effects from methanol if their blood methanol is greater than 0.62 mg/L (0.25 background + 0.37 exposure to reference level). Because a large portion of the population has a background level of methanol above 0.62 mg/L according to Table 3-1 in EPA's assessment, the implication is that many Americans are at risk from their own production of methanol and/or from dietary exposure.

More importantly, Dr. Clark states that the Agency feels that ignoring the background levels of methanol is appropriate because the Agency ran the PBPK model both including and excluding background levels and the results did not differ significantly (<1%) with regard to results that form the basis of the reference levels. However, this argument expressed by the Agency indicates a misunderstanding of the issue in controversy.

We believe it is accurate to say that the rats that serve as the basis of the model had low methanol background levels relative to the very large doses of methanol to which they were exposed.

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[6] Copies of the letter to Dr. Anastas of EPA and EPA's May 27, 2011 reply are included in Appendix B of these comments.

Consequently, the resulting blood levels of methanol in the rats[7] can be ascribed almost entirely to the exogenous exposure of methanol, and that the developmental effects demonstrated in the study can be ascribed to the blood level of methanol, a very high percentage of which was the result of the exogenous exposure. Therefore, it is not surprising that running the PBPK model with and without the background exposure resulted in less than a 1% difference in the modeling results.

The conceptual problem comes, we believe, when EPA applies this reasoning to humans. Assuming that the rat is a proper model for human effects from methanol exposure (a matter we dispute later in these comments), the result of the assessment is a set of recommended reference levels which, in EPA's approach, refer to exogenous exposures, not blood levels of the chemical. If humans had low background levels relative to the proposed reference levels, external exposure of methanol would be the major contributor to blood levels of methanol and endogenous levels would be of no consequence. However, humans have relatively high background levels of methanol compared to EPA's proposed reference levels, produced by the body from processes that do not involve exogenous exposures to methanol.

Exogenous exposures add to these blood levels, and there is no evidence that it is only the exogenous exposures rather than the total blood levels that are of concern, if one uses the rat studies as one's basis. In other words, an adherence to the animal data on which the Agency based its calculations would suggest that the reference levels should be for total blood levels of methanol, not just for the exogenous exposure to methanol. Of course, if this were the case, new issues of the validity of the reference levels would be raised, as discussed further below.

By analogy, according to at least the layman's understanding of cholesterol (about which we have no special knowledge), for many humans the amount of cholesterol that is contained in the food they eat has little correlation with the amount of cholesterol in their bodies. Consequently, for many people dietary control of cholesterol is not a successful way to control the perceived threat of cholesterol levels in the body, and pharmaceutical intervention is necessary for those people to control cholesterol levels. To say that only the cholesterol that one eats (exogenous cholesterol) constitutes the threat would be to ignore the perceived threat of cholesterol no matter what its source—endogenous or exogenous.

Similarly, EPA has presented no evidence that background blood levels of methanol are safe and exogenous levels of methanol in contrast are toxic. The rat data do not demonstrate this because the rats did not have high background levels of methanol relative to the exogenous dose, and the alternative modeling approach that results in a 1% difference in results (leading to almost identical reference levels) provides no further enlightenment.

[7] Fig. B-13, B-14, ranging from 3.6 to 2832 mg/L

If total blood levels of methanol are the point of concern, as we suggest is the logical result of the animal studies that EPA has chosen to rely upon, then this means that a large percentage of the American public are at risk of developmental effects simply from the methanol they produce naturally, even if they never receive any exogenous exposure to methanol. Then, foods they normally consume could add substantially to this risk, based on data regarding the amount of methanol contained in foods. This presumed and unsubstantiated risk to the American public certainly raises serious questions of whether the animal model and the reference level methods of calculation are appropriately applied in the case of methanol. Certainly there is a serious question of whether they are relevant to humans.

We believe that EPA must confront this dilemma: either EPA's reference levels are correct and consequently most people are at risk from their own production of methanol, or the recommended reference levels are incorrect in suggesting that persons exposed to greater than this level are at risk.

In her letter, Dr. Clark indicates that EPA has included a specific charge question related to EPA's approach to background in the list of question s for the external peer review. However, this question reads:

> ***Please comment on the scientific justification for the subtraction of background levels of methanol from the data in relation to the quantification of noncancer risks.***

Given the controversy surrounding this approach to background, this question, drafted earlier in the process, now seems inadequate. We recommend that that EPA ask more specific questions of the Peer Review Panel:

1. Does an exposure above the RfD or RfC have the same impact on a person with a background/endogenous blood level of methanol of 0.25 mg/L as a person with 4.7 mg/L?
2. If endogenous blood methanol level has been determined to be up to 4.7 mg/L, should the RfC and RfD be set at levels that result in blood methanol levels below 4.7 mg/L for some of the population?
3. If exposure above the RfC or RfD represents a risk for persons with a background/endogenous of 0.25 mg/L, are people with a background level above this at risk even without exogenous methanol exposure?

III. B. 2. EPA APPEARS TO IGNORE THE SIGNIFICANT PRESENCE OF METHANOL IN FOOD

In the draft assessment, it is unclear whether methanol in food is considered background or exogenous. If methanol is produced in the body from a food source not containing methanol, it appears to be considered endogenous/background and therefore non-toxic. However, it remains to be clarified whether the methanol present in food and transferred to the blood stream is considered part of the background level or whether it is considered exogenous and thereby subject to the reference dose and potentially toxic. The answer to this question would appear to a member of the public as being very important, but, of course, the methanol in the blood stream as a result of food, whether considered exogenous or endogenous, should be, in our view, treated as equivalent toxicologically

This labeling of food methanol as either exogenous or endogenous becomes an issue only because EPA insists on treating exogenous and endogenous differently and because the levels of methanol in food are significant enough in comparison to the recommended reference level to make a big difference in presumed risk. All this makes EPA's choice of a reference level highly suspect.

The EPA assessment's apparent assumption that any level of endogenous/food-derived methanol is non-toxic, but that small amounts of methanol from drinking water or air (and maybe that present in food) may cause adverse developmental effects can be illustrated further by looking at consumption of orange juice and alcoholic beverages. Using the data contained in the recent methanol assessment by National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (CERHR)[8] and summarized in Appendix A of these comments, the proposed RfD of 0.4 mg/kg/day could be exceeded by drinking 8 oz. of orange juice or 2 beers or 3 oz. of wine. It seems problematic that one federal agency would advocate consumption of fruits for a healthy diet, while another agency proposes to say that such exposure may lead to a developmental risk.

At EPA's May 26th Listening Session for methanol ("Listening Session"), we explained that the above orange juice estimate was based on CERHR's reported mean level of 140 mg/L methanol. The IRIS staff seemed surprised by this high level, and suggested that a level that high must be associated with non-pasteurized orange juice only. They mentioned that the Federal Centers for Disease Control recommends drinking only pasteurized orange juice. The EPA staff's figure of pasteurized orange juice was only 7 mg/L. They implied that a level this low would suggest that consumption of pasteurized orange juice in normal amounts would not cause a person to exceed the recommended reference dose for methanol.

However, our further research shows that EPA's reliance on the 7 mg/L figure is not justified and that the concern remains that normal food intake can cause someone to exceed the

[8] NTP-CERHR Monograph on the Potential Human Reproductive and Development Effects of Methanol, 2003 NIH Publication No. 03-4478.

recommended reference dose. Methanol forms in juices and some other foods due to the unavoidable action of naturally occurring enzymes with pectin in the cell walls. Thus, the amount of methanol that is formed will vary widely occurring to the maturity of the fruit or vegetable, the amount of enzyme present, and the amount of time that the enzyme has to react with pectin.

A wide range in the level of methanol in orange juice has been reported; the CERHR reported values from 1 to 640 mg/L, with a mean of 140 mg/L. The CERHR report does not make the distinction between pasteurized and non-pasteurized juice. We found only 2 reports which discussed levels of methanol contained in pasteurized orange juice. Lund et al. [9](1981) reported little difference between fresh and canned orange juice. They reported a range of 11 to 80 mg/L in fresh and 12-60 mg/L in canned orange juice. They also measured methanol in laboratory reamed, fresh commercial processer extracted, or freshly canned processor extracted orange juice from Valencia oranges and reported values 11, 37, and 25 mg/L respectively. The UK MAFF (1993) conducted a survey of methanol in orange and grapefruit juice. They reported ranges of 7-92 mg/L in fresh squeezed orange juice, 78-132 mg/L in commercial fresh juice, and 1-30 mg/L in date of purchase pasteurized orange juice, with a mean of 7 mg/L. Apparently, EPA picked the mean value of this one report (7 mg/L) as the appropriate comparison level, but as one can see, methanol levels in orange juice vary over a large range, and pasteurization does not always mean low methanol levels. In addition, many people do drink freshly squeezed, un-pasteurized orange juice, and EPA's recommended reference dose should not be established without acknowledgement of this common exposure scenario.

Further calculations are shown in Appendix A to these comments. Greizerstein et al. 10(1981, reported in CERHR) reported 6-27 mg/L methanol in beer and 96 -329 mg/L in wine. As shown in the appendix, consumption of 3 oz of wine with the level of 329 mg/L would exceed the proposed RfD.

An unpublished and unreviewed FDA analysis estimated 90th percentile exposures to methanol resulting from intake of untreated fruit juice and wine and use of DMDC (a preservative often used in alcoholic beverages that decomposes to methanol); they estimated the 90th percentile daily intake of methanol from these sources to be 1.0 mg/kg/day (DiNovi, 1996). Thus the FDA estimated methanol exposure from fruit juice and wine to be 2.5 times the proposed RfD of 0.4 mg/kg/day. In its comments in March of 2010 on the original draft assessment, the Calorie Control Council noted that the FDA has assigned a safe level of dietary methanol exposure to be 7.1 to 8.4 mg/kg body weight/day, or approximately 426 to 504 mg/person/day for a 60 kg adult.

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[9] Lund E, Kirkland C, Shaw P. Methanol, ethanol and acetaldehyde contents of citrus products. J Agric Food Chem 1981;29:361-366.

[10] Greizerstein HB. Congener contents of alcoholic beverages. J Stud Alcohol 1981;42:1030-1037.

Further, according to the FDA (1988), "An adult human can metabolize up to 1500 milligrams of methanol per hour with no adverse symptoms or effects."

In summary, EPA cannot assume that endogenous methanol and methanol contained in food can be treated differently toxicologically from exogenous methanol or that small increments of exogenous methanol are toxic while large amounts of endogenous methanol in the same person are benign. EPA needs to re-calculate its recommended reference levels, taking endogenous/background levels of methanol into account. This is reinforced by the recent recommendation to EPA by the National Research Council regarding the IRIS file on formaldehyde to take the endogenous levels of formaldehyde into account in assessing the health effects.[11] Toxicologically, there is no reason to distinguish methanol from formaldehyde in this regard.

IV. Mode of Action and the Appropriate Animal Model

In the draft assessment, the RfC is derived from rodent developmental toxicity (NEDO, 1987, Rogers et al., 1993) and the RfD is derived by PBPK estimation of an equivalent oral dose to the RfC. The mode of action (MOA) section asserts that a mechanism is not known. In light of recently published research by Dr. Peter Wells, EPA needs to reevaluate the appropriate animal model for deriving the reference levels because Dr. Wells' research shows that either the rodent development effects from methanol exposure are irrelevant to human risk, or at the very least, humans are much less susceptible to development effects from methanol exposure than rodents. The IRIS assessment must account for the role of reactive oxygen species (ROS) in rodent developmental toxicity from methanol and its role in human risk.

IV. A. The Center for the Evaluation of Risks to Human Reproduction (CERHR) reached very different conclusions.

EPA fails to explain why its analysis differs so greatly from the CERHR's recent assessment of methanol[12]. The CERHR document translates to an RfD of at least 50 mg/kg/day versus EPA's recommended RfD of 0.4 mg/kg/day. While EPA in its assessment decided that the mode of action is not known, the CERHR considered the mechanism to be more likely related to methanol than metabolites, such as formaldehyde or formate. The CERHR

[11] National Research Council, National Academy of Sciences, Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde, 2011, Summary, page 3; Toxicokinetics, page 23.
[12] Footnote 8

concluded there was little likelihood of developmental effects from methanol exposures unless they were sufficient to cause human blood methanol levels to be greater than 10 mg/L. Based on human exposure data, blood methanol levels above 10 mg/L would only be caused by exposure to greater than 50 mg/kg methanol orally or by inhalation of more than 200 ppm methanol for at least 6 hours (100 fold higher than RfD or RfC). The CERHR's assessment also concluded that methanol from foods is unlikely to be of a concern. Given CERHR's specialized expertise in developmental effects, EPA should carefully explain how it could reach such very different conclusions.

IV. B. EPA LEAVES OUT RECENT STUDIES THAT CHALLENGE THE AGENCY'S BASIS FOR THE REFERENCE LEVELS.

IV. B. 1. METABOLISM IN RABBITS IS MORE SIMILAR TO HUMANS' THAN THAT OF RODENTS

EPA's reliance on rodent developmental toxicity for human risk assessment has been called into question by recent studies conducted by Dr. Peter Wells of the University of Toronto conducted under an unrestricted grant from the Methanol Foundation.

This research was undertaken at the suggestion of the EPA staff (including Dr. John Rogers) in discussions at the beginning of the IRIS review of methanol in 2005—making it all the more important that EPA assess the results of this research and incorporate them, as appropriate, in the draft IRIS assessment. As noted in the draft methanol assessment, metabolism of methanol in rodents is different from other mammals. One of Dr. Wells' recent publications (previously submitted to the EPA IRIS office) demonstrated that the metabolism of methanol in rabbits more closely resembles that of primates (measured in monkeys) than metabolism in rodents (mice). The abstract is provided below.

> ***Sweeting, J. N., Siu, M., McCallum, G. P., Miller, L. and Wells, P. G. Species differences in methanol and formic acid pharmacokinetics in mice, rabbits and primates. Toxicology and Applied Pharmacology 247(1): 28-35, 2010.***
>
> *"Methanol (MeOH) is metabolized primarily by alcohol dehydrogenase in humans, but by catalase in rodents, with species variations in the pharmacokinetics of its formic acid (FA) metabolite. The teratogenic potential of MeOH in humans is unknown, and its teratogenicity in rodents may not accurately reflect human developmental risk due to differential species metabolism, as for some other teratogens. To determine if human MeOH metabolism might be better reflected in rabbits than rodents, the plasma pharmacokinetics of MeOH and FA were compared in male CD-1 mice, New Zealand white rabbits and cynomolgus monkeys over time (24, 48 and 6 h, respectively) following a single intraperitoneal injection of 0.5 or 2 g/kgMeOHor its saline vehicle. Following the high dose, MeOH exhibited saturated elimination*

kinetics in all 3 species, with similar peak concentrations and a 2.5-fold higher clearance in mice than rabbits. FA accumulation within 6 h in primates was 5-fold and 43-fold higher than in rabbits and mice respectively, with accumulation being 10-fold higher in rabbits than mice. Over 48 h, FA accumulation was nearly 5-fold higher in rabbits than mice. Low-dose MeOH in mice and rabbits resulted in similarly saturated MeOH elimination in both species, but with approximately 2-fold higher clearance rates in mice. FA accumulation was 3.8-fold higher in rabbits than mice. Rabbits more closely than mice reflected primates for in vivo MeOH metabolism, and particularly FA accumulation, suggesting that developmental studies in rabbits may be useful for assessing potential human teratological risk."

IV. B. 2. SENSITIVE RODENTS ARE NOT A GOOD MODEL FOR EFFECTS IN HUMANS

Recent research has demonstrated that some rodents are sensitive to methanol while other rodents are not affected. This raises additional questions about whether EPA's choice of a sensitive rodent strain as a model for predicting effects in humans is appropriate.

In addition to the metabolism of methanol being different in rabbits and rodents, further studies have demonstrated that methanol does not cause developmental toxicity in all strains of mice. Furthermore, it was also shown that methanol does not cause developmental effects in rabbits; there was no developmental toxicity in either C3H mice or New Zealand rabbits exposed to methanol. The abstract is provided below:

Sweeting, J. N., Siu, M., Wiley, M. J. and Wells, P. G. Species- and strain-dependent teratogenicity of methanol in rabbits and mice. Reproductive Toxicology: 31(1): 50-58, 2011.

Estimates of human risk for developmental toxicity of methanol (MeOH) are based on studies in rodents, which unlike humans use catalase to metabolize MeOH. Rabbits, like humans, may largely use alcohol dehydrogenase (ADH), and more accurately than rodents reflect primate MeOH and formic acid (FA) pharmacokinetic profiles. Here we show that New Zealand white rabbits and one strain of mouse (C3H) are resistant to MeOH teratogenicity, whereas C57BL/6J mice are susceptible. Neither rabbits nor mice were susceptible to the acute MeOH toxicity observed in humans. The strain-dependent teratological susceptibility in mice could not be explained by differences in MeOH or FA disposition, nor could the resistance of rabbits, which exhibited more prolonged FA accumulation, suggesting that different mechanisms underlie MeOH

teratogenesis and the FA-mediated acute toxicity in humans. It is not clear if the human risk for MeOH developmental toxicity can be accurately estimated using sensitive rodent strains.

IV. B. 3. EPA DOES NOT ADDRESS THE ROLE OF REACTIVE OXYGEN SPECIES IN RODENTS

Experiments from the University of Toronto demonstrate that reactive oxygen species (ROS) are likely involved in the developmental toxicity of methanol in rodents.

Embryos in culture from genetically modified mice that contain human catalase (hcat) were almost completely protected from methanol-induced embryopathic effects, while methanol was more embryopathic in embryos in culture from homozygous C3Ga.Cg-Catb/J acatalasemic (aCat) mice than from WT C3H mice. These data suggest that reactive oxygen species are likely involved in the embryopathic mechanism of methanol. The abstract is provided below:

Miller, L. and Wells, P. G. Methanol embryopathies in embryo culture in transgenic mice expressing human catalase, and mutant catalase-deficient mice. Toxicology and Applied Pharmacology: 252: 55-61, 2011.

The mechanisms underlying the teratogenicity of methanol (MeOH) in rodents, unlike its acute toxicity in humans, are unclear, but may involve reactive oxygen species (ROS). Embryonic catalase, although expressed at about 5% of maternal activity, may protect the embryo by detoxifying ROS. This hypothesis was investigated in whole embryo culture to remove confounding maternal factors, including metabolism of MeOH by maternal catalase. C57BL/6 (C57) mouse embryos expressing human catalase (hCat) or their wild-type (C57 WT) controls, and C3Ga.Cg-Catb/J acatalasemic (aCat) mouse embryos or their wild-type C3HeB/FeJ (C3H WT) controls, were explanted on gestational day (GD) 9 (plug=GD 1), exposed for 24 h to 4 mg/ml MeOH or vehicle, and evaluated for functional and morphological changes. hCat and C57 WT vehicle-exposed embryos developed normally. MeOH was embryopathic in C57 WT embryos, evidenced by decreases in anterior neuropore closure, somites developed and turning, whereas hCat embryos were protected. Vehicle-exposed aCat mouse embryos had lower yolk sac diameters compared to C3H WT controls, suggesting that endogenous ROS are embryopathic. MeOH was more embryopathic in aCat embryos than WT controls, with reduced anterior neuropore closure and head length only in catalase-deficient embryos. These data suggest that ROS may be

involved in the embryopathic mechanism of methanol, and that embryonic catalase activity may be a determinant of teratological risk.

IV. C. DR. WELLS' RESEARCH IS DIRECTLY RELEVANT FOR HUMAN HAZARD ASSESSMENT

Dr. Wells' research is highly relevant for human hazard assessment, and EPA's assessment of methanol and its recommended reference levels must therefore be significantly revised to accommodate it.

At the Listening Session, EPA questioned whether Dr. Wells' research, using a high dose in mice administered by the intraperitoneal (ip) route in a single day was relevant to the evaluation of developmental effects found from lower-dose repeated exposure experiments in rats, which are the basis for EPA's assessment. In response, it is important to understand that Dr. Wells' research was directed toward two key issues:

- **The role of oxidative stress in the mechanism of methanol teratogenicity; and**
- **Whether human risk might be predicted by more accurately by an animal model that metabolized methanol more similarly to humans than rodents.**

One element of the EPA staff's question is why mice rather than rats were an appropriate species to address these issues. Mice were chosen over rats as the rodent model because:

1. Methanol teratogenicity has been most extensively characterized in vivo and in embryo culture using mice, including both the teratological outcomes and the underlying mechanisms. Mice accordingly provide the best foundation upon which to base mechanistic studies; and

2. For the most definitive evaluation of mechanisms, genetically modified animals with alterations in potentially critical proteins or pathways generally provide more reliable information than the use of chemical modulators, which usually alter multiple proteins or pathways, many of which may be unappreciated, potentially confounding the interpretation of results. An extensive array of mouse strains with genetic alterations in specific proteins or pathways is already available and rapidly expanding, particularly with respect to reactive oxygen species (**ROS**) and oxidative stress. In Dr. Wells' studies, these models included mice deficient in catalase, mice expressing enhanced levels of catalase, and mice lacking repair of oxidative DNA damage. These genetically modified models are not available in rat strains.

Although rats have technical advantages over mice, including providing larger blood and tissue volumes for repetitive sampling to measure drug concentration profiles and other parameters, they generally do not offer any scientific advantage over mice. Nevertheless, there can be

substantial differences among rat strains, as there are among mouse strains, in their susceptibility to certain teratogens. Accordingly, a particular rat strain may be very different from a particular mouse strain. However, considering susceptible strains, rats and mice generally exhibit similar dose-response curves, although the curves may be shifted, with one species exhibiting relatively more or less sensitivity than the other species. For example, both rats and mice are susceptible to the teratogenic effects of the ROS-initiating antiepileptic drug phenytoin, although a higher range of doses is required in rats.

The intraperitoneal (ip) route of administration and an acute, high dose of methanol were used because:

1. The ip route, as opposed to inhalation or oral intake, is essential for ensuring that the dose delivered is as consistent as possible, in part for a precise determination of pharmacokinetic parameters, particularly across species like mice, rabbits and monkeys. Equally importantly, the resulting consistency in embryonic exposure allows a more reliable interpretation of results from mechanistic studies relating molecular changes to teratological outcomes;

2. The high dose of methanol, well above the lethal dose in humans, was based upon published studies of methanol teratogenicity in mice, and retained in rabbits and monkeys to ensure that any teratogenic potential was not missed due to an insufficiently high dose, as well as to facilitate cross-species comparisons;

3. Methanol was administered acutely rather than chronically to avoid the complicating secondary biochemically changes that accumulate with chronic exposures, which can confound the interpretation of data relevant to the mechanisms of teratogenic initiation. The high methanol dose employed assured that any potential for initiating oxidative stress would not be missed by the use of an acute dose. This regimen produced teratogenic effects similar to those reported in the literature with other dosing regiments in a susceptible mouse strain, so any teratologically relevant ROS involvement would be evident under these conditions; and

4. In mouse embryo culture, the concentrations of methanol were based in part upon published studies, and chosen to span the range from no effect to maximal embryopathic effects in the mouse model (124-187 mM, or 2-6 mg/ml). This range is essential for testing potential mechanisms of methanol embryopathies by determining the modulatory effects of probes like free radical spin trapping agents.

A second element of the EPA staff's question at the Listening Session was whether information derived from a high dose single day study is relevant to developmental effects found in lower-dose repeated experiments and to hazard assessment for humans. In Dr. Wells' in vivo studies in several strains of adult male mice, the mean peak plasma concentration following a 2 g/kg dose approximated 80 mM. In his teratological studies, a divided dose of 4 g/kg was administered, likely approximating a maternal peak plasma concentration of 160 mM, which is above the

minimal embryopathic concentration (124 mM) of methanol used in his embryo culture studies. Teratological outcomes in Dr. Wells' in vivo model were similar to those reported in the published literature using other models and dosing regimens. Although doses required to produce teretological effects may differ between rats and mice or between single high doses and lower repeated doses, Dr. Wells' research clearly establishes that increased ROS is an important component of methanol-induced developmental effects. In the absence of increased ROS, methanol is not likely to cause developmental effects.

Dr. Wells' research demonstrates that some strains of mice, as well as rabbits, are resistant to the developmental effects of methanol and that differences in ROS contribute to those differences. Since the metabolism of methanol in humans does not produce increased levels of ROS, developmental effects in sensitive rodent strains should not be taken as the point of departure for human risk assessment (RfD, RfC) without adjusting for differences in metabolism and the production of ROS. EPA's assessment and its recommended reference levels therefore must be significantly altered to take these new research results into account.

V. SUMMARY

EPA has arbitrarily decided to establish reference levels to identify risks ONLY for exposure to methanol that increases the body burden of methanol or its metabolites. The recommended reference levels represent a very small addition to the average person's body burden of methanol and by implication suggest that half the population is at risk from their own background level of methanol. EPA incorrectly supports its decision to ignore the naturally-occurring background levels of methanol in human blood by citing the results of its PBPK modeling. While the increment of a reference level dose of methanol is a small percentage of the average background blood level in humans, the intake of certain common foods can easily exceed this recommended reference level. The implications of the EPA assessment, then, are that most Americans are already at risk of developmental effects from their naturally-produced methanol, even without any exposure to external methanol, and that the consumption of some foods will put them at even greater risk. These issues with the reference levels are sufficient in and of themselves to warrant a re-assessment by EPA of the basis and methods of calculating reference levels for methanol.

In addition, EPA's recommended reference values are based on developmental toxicity studies in rodents. Recent published research by Dr. Wells indicates that adjustment to the point of departure or the endpoint of concern is needed. The research indicates that methanol metabolism by catalase in rodents results in developmental effects caused by reactive oxygen species (ROS), not by methanol or another metabolite. Humans and most non-rodents do not metabolize

methanol using catalase and ROS are not produced. Therefore, either the rodent developmental effects from methanol exposure are irrelevant to human risk, or at the very least, humans are much less susceptible to developmental effects from methanol exposure. The IRIS assessment must account for the role of ROS in rodent developmental toxicity from methanol and its role in human risk.

For these reasons, the current approach for determining reference levels for methanol cannot be supported, and the assessment must be changed and then subjected to public comment and peer review again. These necessary changes are too fundamental to the assessment document for them to be made without the altered document's undergoing a complete review by scientists outside the Federal Government and an independent peer review panel. The Methanol Institute has urged EPA to make these changes to the document BEFORE proceeding with public comment and peer review; however, should EPA decide otherwise, then once the changes are made, a completely new review cycle will be absolutely necessary. To do otherwise would be inconsistent with the Agency's commitment to peer review of all major decisions documents.

Should EPA proceed to peer review without changing the document, as EPA has recently indicated it intends to do, EPA should at a minimum address these fundamental criticisms in a written document. That document should be provided to the public for written comment by Dr. Wells and other outside scientists with sufficient lead time so that these written comments can be placed before the peer reviewers BEFORE they are required to draft their initial comments on the assessment document. In addition, more detailed and focused charge questions should be constructed and presented to the peer review panel to address these critical issues adequately.

We urge EPA not to rush ahead with the peer review of an incomplete document, but to pause and address these issues fully in its assessment effort BEFORE peer review of the assessment.

APPENDIX A

POSSIBLE METHANOL EXPOSURES FROM BEVERAGES RELATIVE TO RFD: REPORTED LEVELS OF METHANOL IN FOODS (CERHR)

Reported Level: Orange and grapefruit juice – 1-640, mean 140 mg/L

Exposure calculations for orange juice (OJ):

1 oz = 30 ml

methanol/oz of OJ (140 mg/L * .03 L/oz) = 4.2 mg/oz.

Adult

methanol/oz OJ/kg BW (4.2 mg/oz /70 kg adult) = 0.06 mg/kg/oz

oz OJ to exceed proposed RfD = RfD (0.4 mg/kg)/0.06 mg/kg/oz = **6.6 oz.**

Child

methanol/oz OJ/kg BW (4.2 mg/oz /20 kg adult) = 0.21 mg/kg/oz

oz OJ to exceed proposed RfD = RfD (0.4 mg/kg)/0.21 mg/kg/oz = **1.9 oz.**

Reported Level: Beer- 6-27 mg/L

Exposure calculations for beer:

Methanol/oz beer (27 mg/L * 0.03 L/oz) = 9.87 mg/oz

Methanol/ kg BW /oz consumed = 9.87 mg/oz /70 kg) = 0.012 mg/kg/oz

Oz beer to exceed RfD (0.4 mg/kg / 0.012 mg/kg/oz = **33 oz**

Reported Level: Wine – 96-329 mg/L

Exposure Calculations for Wine

Methanol/oz wine (329 mg/L * 0.03 L/oz) = 0.81 mg/oz

Methanol/ kg BW /oz consumed = 0.81 mg/oz /70 kg) = 0.14 mg/kg/oz

Oz wine to exceed RfD (0.4 mg/kg / 0.14 mg/kg/oz = **2.85 oz**

APPENDIX B

SIMULATIONS OF 24 H EXPOSURE OF HUMANS TO METHANOL AT PROPOSED RFD AND RFC

Teresa L. Leavens, PhD

Sunday, May 15, 2011

The US EPA physiologically based pharmacokinetic model, which was provided with the release in January of 2010 with the draft IRIS risk assessment for methanol, was used to run simulations for a 24 h exposure of humans to methanol at the proposed RfC of 2 ppm for inhalation exposure and proposed RfD of 0.4 mg/kg/day for oral exposure. The simulations used the default human parameters provided in the model for a standard 70 kg man and were performed with background concentrations set to zero in order to show just the results of the exogenous exposure. The inhalation exposure was a continual 24 h exposure and used the resting values for ventilation and perfusion. The oral bolus dose was a single dose of 28 mg to a 70 kg man, while the drinking water was compared according to the 3 methods described in the draft IRIS document (Section B.3.6, Figure B-24 and Table B-9). The 3 methods included a constant infusion for either 12 h or 24 h for a total of 28 mg/day for a 70 kg man, or 6 bolus doses of varying amounts and ingestion intervals for a total of 28 mg/day for a 70 kg man. For the inhalation and infusion simulations, the steady state concentrations in blood from the exogenous methanol exposures were compared to the background methanol concentration, which EPA has set at 2 mg/L. For the single oral bolus and the multiple drinking water boluses, the maximum concentrations in the blood during the 24 h period were compared to the background methanol concentration. Table 1 below summarizes the blood concentrations of methanol from each exposure, while Figure 1 shows the simulated blood concentration from a 2ppm inhalation exposure to methanol, and Figure 2 shows the simulated blood concentrations from the single oral bolus exposure and 3 drinking water scenarios.

Table 1. Comparison of the Cmax from Inhalation, Oral Bolus, and Drinking Water

Exposure Route	**Cmax (mg/L)[a]**	
	(mg/L)	% Background[b]
Inhalation 2 ppm	0.0421	2.1
Oral Bolus 0.4 mg/kg	0.2640	13.2
Drinking water (12 hr infusion for total of 0.4 mg/kg/day)	0.0551	2.8
Drinking water (24 hr infusion for total of 0.4 mg/kg/day)	0.0275	1.4
Drinking water (6 bolus dose pattern for total of 0.4 mg/kg/day)	0.0788	3.9

[a]For inhalation and drinking water modeled as infusion, the listed concentration is the steady state concentration during exposure, and the maximum concentration reached for the oral bolus and the drinking water modeled as 6 bolus doses. These simulations were performed assuming zero background concentrations.

[b]EPA lists background methanol concentrations as 2 mg/L

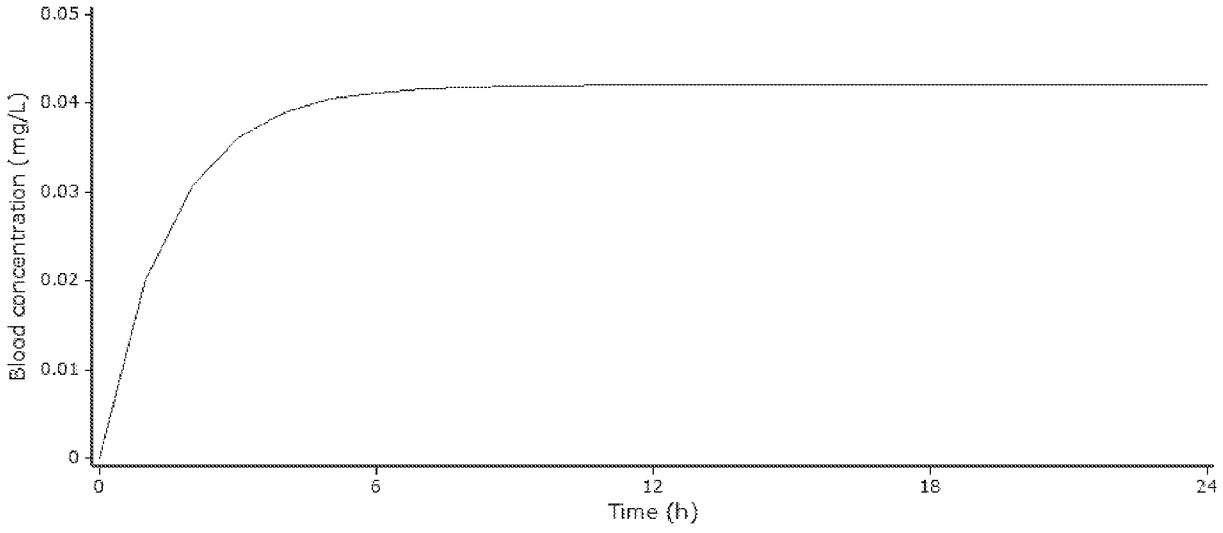

Figure 1. Inhalation exposure to 2 ppm Methanol

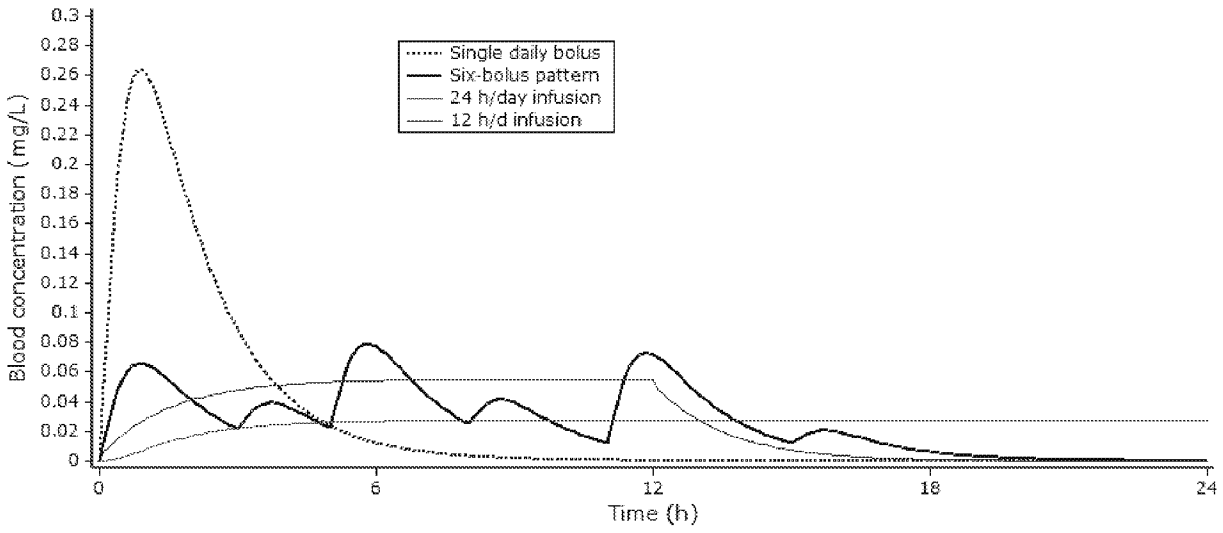

Figure 2. Oral exposures to 0.4 mg/kg/day methanol

APPENDIX C

LETTER EXCHANGE BETWEEN THE METHANOL INSTITUTE AND EPA'S DR. PAUL ANASTAS & DR. REBECCA CLARK (ATTACHED)

Penthouse Level
Suntec Tower 3
8 Temasek Blvd
Singapore 038988
Tel: +65 6866 3238

Methanol Institute

124 South West Street
Suite 203
Alexandria, VA 22314
Tel: 703.248.3636

April 28, 2011

Dr. Paul Anastas
Assistant Administrator for
Research and Development
1200 Pennsylvania Avenue
U.S. Environmental Protection Agency
Washington, DC 20004

RE: Withdrawal of the Noncancer IRIS Assessment of Methanol

Dear Dr. Anastas:

We are writing to request that you withdraw the noncancer IRIS assessment of methanol from its current public and peer review cycle. In our view, the current assessment is so fundamentally flawed that to proceed with peer review of the document would be meaningless, and in fact, counterproductive to good scientific process.

As you know, the current noncancer IRIS assessment is identical to the December, 2009 IRIS assessment of methanol released in January, 2010, except that the cancer sections of the assessment have been removed. Your staff has chosen to republish for public comment and peer review these identical pages without any alteration to reflect the changes in the scientific landscape for methanol in the intervening period. These changes require fundamental changes in the analysis of the methanol assessment which promise to radically alter the conclusions of the assessment, as we briefly outline below.

When asked why EPA has chosen to proceed in this manner, EPA staff have responded that this approach allows the Agency to avoid putting the assessment and the new science through the intra- and inter-agency review processes, thereby avoiding delay. The intent is to put the current draft (known by EPA staff to be flawed) through peer review, and THEN make the necessary changes to reflect the new science before publication. However admirable the goal of keeping to schedules may be, this desire to stay on schedule should not trump good scientific processes that are designed to assure the integrity of the resulting assessment.

We realize that your office often receives requests to delay or re-do your IRIS assessments, so let us explain why we believe that the developments since December 2009 fundamentally undermine the core elements of the current assessment and therefore necessitate a re-working NOW of the assessment rather than later after peer review:

The current draft assessment of noncancer effects of methanol bases its RfC and RfD conclusions on developmental effects in rodents AND makes these calculations on the basis of a PBPK model that DOES NOT take background levels of methanol into account.

The events since December 2009 that fundamentally challenge this approach to the assessment of noncancer effects are as follows:

- On April 8, 2011 the National Academy of Sciences released its report on the IRIS assessment of formaldehyde. Among the Academy's conclusions was the following: ***"The endogenous production of formaldehyde complicates the assessment of the risk associated with formaldehyde inhalation and remains an important uncertainty in assessing the additional dose received by inhalation, particularly at sites beyond the respiratory tract."*** Such endogenous and food sources of methanol also complicate an assessment of methanol. Human background levels appear to range from 0.4 and 4.0 mg/L. This range overlaps the proposed reference concentrations. For example, an 8 ounce glass of orange juice contains enough methanol to exceed the proposed reference concentration. Ignoring this problem of background is not a proper response from EPA any more in the case of methanol than it is in the case of formaldehyde.

- During 2010 and early 2011, the results of 4 years of research by the University of Toronto, instigated at EPA's suggestion, on the relevance of rodents for assessment of the health effects of methanol, were published, and subsequently brought to EPA's attention. These studies demonstrate that: methanol metabolism in rodents is different from primates (monkeys) and rabbits; that developmental toxicity from methanol is restricted to sensitive strains of rodents (no developmental effects in some strains of mice or in rabbits); that rodent metabolism of methanol by catalase produces reactive oxygen species (ROS) that play a large role in the developmental effects; and little ROS are produced in non-rodents, explaining why they are not sensitive to methanol developmental effects. EPA has no plans to point out these studies to the peer review panel, despite the fact that the proposed reference concentrations are based on rodent studies.

- More than a year ago, on March 15, 2010, the Methanol Institute submitted public comments on the current draft that presented information regarding both the relevance of the rodent data for assessment purposes for methanol and the need to take background levels of methanol into account. The Department of Defense submitted similar criticisms of the current draft during the Interagency Review process. **EPA staff have chosen not to respond in any way to either set of comments in the current draft, despite adequate time and opportunity.** EPA's proposed process now requires the Methanol Institute to re-submit these comments and to try to summarize them in 5 minutes before a peer review panel. Such a process may make sense for some issues, but not for such fundamental issues as the relevance of the rodent data to humans and the inadequacy of the PBPK model that was used by EPA staff to arrive at the proposed reference concentrations.

Your office's published process used for the Independent External Peer Review of IRIS files[1] has the following relevant statement of policy:

> *"The EPA takes its responsibility concerning peer review very seriously. EPA recognizes the importance of independent, external peer review in maintaining high standards for the quality of the science and technical products that EPA produces and sponsors. Peer review is an important component of the scientific process that provides a focused, objective evaluation of a draft product. The constructive criticisms, suggestions, and new ideas provided by the peer reviewers stimulate creative thought, and strengthen and confer credibility on the product. Comprehensive, objective peer reviews lead to good science and product acceptance within the scientific community. Thus, peer review insures that the Agency's scientific reports are held to the highest possible standards." (Page 1).*

[1] Policy and Procedures for Conducting IRIS Peer Reviews" (2009) (http://www.epa.gov/iris/pdfs/Policy_IRIS_Peer_Reviews.pdf).

As an example of how meaningless a peer review of the current draft assessment would be, particularly in light of the above policy of your office regarding peer review, the charge questions for the peer review ask: "Please comment on the scientific justification for the subtraction of background levels of methanol from the data in relation to the quantification of noncancer risks." The question is no longer should background levels be subtracted (the NAS has answered this question), but rather "How should the noncancer effects of methanol be quantified when background must be considered? How does one determine the contribution of background levels and exogenous levels to the total dose?" These are difficult questions to answer (which is probably the principal reason that EPA staff chose to subtract the background concentrations). This issue is made even more difficult in light of the Toronto research which shows that the rodent data probably do not provide a relevant starting point for such quantification.

EPA scientists should grapple with these challenging scientific issues BEFORE submitting the IRIS document for peer review, not AFTERWARDS. Otherwise, the peer review would be a worthless exercise. We note that these same issues regarding the assessment were raised in comments submitted by the Department of Defense on the 2009 draft and were ignored by EPA staff before releasing the 2009 draft for public review. This refusal to address legitimate and fundamental scientific comments and to proceed instead to peer review raises serious questions regarding EPA staff's commitment to "fix everything" after peer review and before the assessment becomes final.

We are therefore requesting that you withdraw the current noncancer assessment of methanol from the review cycle in order to allow the EPA staff to incorporate this new science and the new guidance from the Academy BEFORE resubmitting the assessment to the review cycle. We believe that the summary of the evidence provided in this letter should be sufficient for you, in consultation with your staff that are familiar with the details of what we present here, to decide to honor our request. However, should you wish to have a fuller presentation of the arguments for withdrawal, we suggest that you instruct your staff NOT to schedule the peer review of the methanol document as this time and then allow us and your staff to discuss these matters, hopefully in your presence, at the Listening Session now scheduled for May 26, 2011.

EPA staff have indicated to us that our March 15, 2010 and listening session comments on the cancer portion of the original draft assessment were very helpful and in part led to your staff's serious concerns regarding the Ramazzini Institute's methanol study. These concerns ultimately precipitated the Agency's decision to first put the entire methanol assessment on hold last June, and the more recent announcement that a full Pathology Working Group review of the methanol – and other studies – is now underway at the Italian lab, and that the cancer portion of the assessment remains on hold until this review has been completed. We certainly commend the EPA for taking these very appropriate steps to ensure that the best available science is used for the cancer portion of the assessment, and are suggesting here that no less an action is required to ensure the scientific integrity of the non-cancer portions of the methanol assessment.

Thank you for your attention to this important matter. We would like to meet with you to discuss this request in more detail and will call your office in a few days to seek a mutually satisfactory time for such a meeting.

Sincerely,

Gregory Dolan
Executive Director
Americas/Europe

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

MAY 27 2011

OFFICE OF
RESEARCH AND DEVELOPMENT

Gregory Dolan
Vice President for
Communications and Policy
Methanol Institute
4100 North Fairfax Drive
Arlington, Virginia 22203

Dear Mr. Dolan:

This is in response to your letter dated April 28, 2011 submitted on behalf of the Methanol Institute in regards to EPA's External Review Draft IRIS Toxicological Review of Methanol (Non-Cancer).

In January, 2010, the IRIS Program released the draft IRIS Toxicological Review of Methanol that addressed both cancer and non-cancer endpoints. As you are aware, a subsequent memorandum from the National Toxicology Program (Malarkey et al., 2010) on the evaluation of tumors in an animal study of methanol reported by the European Ramazzini Foundation (ERF) led EPA to put the assessment review on hold pending further evaluation. Upon further review, EPA concluded that concerns regarding the ERF tumor findings did not affect the assessment of potential non-cancer health effects for methanol, and, thus, EPA is moving forward with external peer review of the draft non-cancer portion of the methanol assessment.

In your letter, you expressed concern about the consideration of recent scientific literature published by the University of Toronto in the draft assessment. EPA is aware of the new literature and evaluated the studies prior to the recent release of the non-cancer assessment for their potential impact on the conclusions of the assessment. Based on the public comments at the May 26, 2011 Methanol Listening Session, EPA agrees that the recent scientific literature published by the University of Toronto should be included in EPA's evaluation of the available scientific literature for Methanol. EPA will develop an addendum to the April 2011 External Review Draft IRIS Toxicological Review of Methanol (Non-Cancer). This addendum will be made publically available as soon as possible and will include study descriptions and analysis of the study data for evaluation by the assessment peer review panel.

You also expressed concerns regarding the consideration of background levels of methanol. In the draft assessment, background levels were accounted for by subtracting them from the dose-response data before a physiologically based pharmacokinetic (PBPK) model was applied. While it is correct that the methanol PBPK model used by EPA does not contain a background parameter, EPA feels this is justified based on testing results that indicated that use of a

background term would have little impact on the benchmark dose used to derive reference levels (see further discussion in the attachment).

The attachment also includes responses to some additional concerns that you raised. I would like to assure you that we very carefully consider comments regarding our assessments. Thank you again for your letter and your continued interest in EPA's IRIS assessment of methanol and the IRIS Program.

Sincerely,

Rebecca Clark
Acting Director

Attachment

cc: Becki Clark
Lynn Flowers
Jeff Gift
Reeder Sams
John Vandenberg
Debra Walsh
Darrell Winner

Attachment

Overview: *A letter dated April 28th, 2011 (ORD-11-000-6811) was submitted on behalf of the Methanol Institute (MI) by Greg Dolan. This attachment discusses the main points of the letter.*

In the MI letter, concerns were expressed regarding the consideration of background levels of methanol. In the draft assessment, background levels were accounted for by subtracting them from the dose-response data before the physiologically based pharmacokinetic (PBPK) model was applied. While it is correct that the methanol PBPK model used by EPA does not contain a background parameter, EPA feels this is justified based on testing results that indicated that use of a background term would have little impact on the benchmark dose used to derive reference levels (see further discussion below).

In reference to endogenous levels and food sources of methanol, the concluding section of the methanol assessment, Section 6, expressly states that "this assessment provides estimates of noncancer risk from oral and inhalation exposures above sources of methanol that contribute to background blood levels." The MI letter states that "Human background levels appear to range from 0.4 and 4.0 mg/L". A slightly broader range is documented in Table 3-1 of the draft methanol noncancer assessment. Section 3.1 of EPA's assessment states that "Table 3-1 summarizes background blood methanol levels in healthy humans which were found to range from 0.25-4.7 mg/L." For individuals of the population in the lower end of this range, exposure to RfC and RfD levels of methanol are predicted by the PBPK model to raise methanol blood levels by more than 150% over their background levels.

With respect to the National Academy of Sciences (NAS) review of a draft IRIS assessment for formaldehyde, the NAS noted endogenous formation of formaldehyde complicates the assessment of [formaldehyde] risk, though the NAS did not answer the question of whether background levels should be subtracted prior to dose-response assessment. Unlike methanol, background levels of formaldehyde (e.g., blood/plasma levels) are difficult to measure and rarely, if ever, reported. Recognizing the question regarding consideration of background, the EPA has included a specific charge question related to EPA's approach for the methanol assessment, for the external peer review panel to address.

The MI letter compared levels of methanol in orange juice to "the proposed reference concentration", though the letter did not provide a citation or indicate what kind of orange juice was being referred to (e.g., pasteurized or unpasteurized, aged or fresh). EPA assumes that the MI either meant to refer to EPA's proposed oral reference dose (RfD) or agree with EPA that the "reference concentration" (RfC) and RfD would result in an equivalent internal (blood) dose. As defined in the first section of the draft methanol assessment, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a

lifetime. According to the FDA, 98% of all juices sold in the United States are pasteurized and the CDC recommendation is that "if it is unclear that a juice has been treated [e.g., pasteurized] to destroy harmful bacteria, avoid drinking it" (www.cfsan.fda.gov/~dms/fttfruit.html). A United Kingdom Food Surveillance Information Sheet for "Methanol in Orange Juice" (http://archive.food.gov.uk/maff/archive/food/infsheet/1993/no17/17orange.htm) indicates that pasteurized orange juice contains an average of 7 mg methanol/kg. At this methanol concentration, a 70 kg person would need to consume approximately one gallon (~128 ounces) per day of orange juice over the course of their entire lifespan to achieve a chronic exposure equivalent to the proposed 0.4 mg/kg/day RfD.

In the MI letter, it is emphasized in boldface font that "EPA staff have chosen not to respond in any way to either set of [MI and Department of Defense] comments [regarding background levels of methanol] in the current draft, despite adequate time and opportunity." This is an unfortunate mischaracterization. EPA considered and responded to all interagency review comments and EPA will carefully consider the comments from the MI, including an EPA response to these and other comments in Appendix B of the final IRIS assessment. Specifically, as part of EPA's response to DOD comments, EPA developed a draft test model that contained a background parameter to investigate the impact of a background parameter on the dose-response estimates for methanol noncancer effects. These investigations are described in Section 3.4.3.2 of the current draft assessment as follows:

> The primary purpose of this assessment is for the determination of noncancer risk associated with exposures that increase the body burden of methanol or its metabolites (e.g., formate, formaldehyde) above prevailing, endogenous levels. Thus, the focus of model development was on obtaining predictions of increased body burdens over background following external exposures. To accomplish this, the PBPK models used in this assessment do not account for background levels of methanol, formaldehyde or formate. In addition, background levels were subtracted from the reported data before use in model fitting or validation (in many cases the published data already have background subtracted by study authors). This approach for dealing with endogenous background levels of methanol and its metabolites assumes that: (1) endogenous levels do not contribute significantly to the adverse effects of methanol or its metabolites; and (2) the exclusion of endogenous levels does not significantly alter PBPK model predictions. There is uncertainty associated with these assumptions. Human data are not available to evaluate whether there is a relationship between background levels of methanol or its metabolites [to adverse noncancer effects]. To test the assumption that the exclusion of endogenous background levels does not significantly alter PBPK model predictions, EPA performed the following alternative analysis using models that incorporate background levels of methanol and its metabolites.
>
> **3.4.3.2.1.Alternative modeling approach – incorporation of background.** If background methanol levels are high enough compared to those which induce metabolic saturation, they may have a significant impact on parameter estimation and hence internal dose predictions. To gauge the impact of background levels on PBPK model predictions of exposure-induced changes in internal doses, alternate (test) versions of the rat and human PBPK models were created which incorporate a zero-order liver infusion term for methanol designed to approximate reported rat and human background levels. Internal dose estimates for various exposure levels obtained from the PBPK models that exclude background up front could then be compared with those from models for which background levels were modeled, but then subtracted for benchmark dose (BMD) modeling. For example, when background levels are included in the PBPK model and the

metric is blood AUC, BMD analysis used the PBPK-predicted difference, AUC(exposed rats) – AUC(control rats), as the dose metric. After obtaining an internal dose point of departure (POD) at a specific effect level for the rat with that metric, the human equivalent internal dose was taken to be POD + AUC(human background). In short the level of effect (above background) was correlated with the internal dose *above* background in the animal, then the human background internal dose was added to the POD obtained with that metric to yield an estimate of the dose when humans would have the same level of effect. The two PBPK modeling approaches (i.e., including or excluding background levels in the PBPK model) did not differ significantly (<1%) with respect to their internal dose point of departure (POD, level above background) estimates from the principal rat noncancer studies. HEC and HED estimates from the principal rat noncancer studies using the human PBPK model with background included were only about 14% lower than those estimated using the human PBPK model with background excluded. Because the more complex PBPK modeling required to include background levels was estimated to have a minimal impact on dose extrapolations, the use of simpler methanol models that do not incorporate background levels is considered adequate for the purposes of this assessment.

The MI letter states that their concerns regarding the points above, and other concerns such as the time for presentation of public comments during the peer review process, result in their request to withdraw the current noncancer assessment of methanol. The EPA welcomes these and other comments and will fully consider the scientific and procedural issues raised.

References

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